Vol 11, Special Issue 1, 2019

INTERNATIONAL JOURNAL OF APPLIED PHARMACEUTICS

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

ISSN-0975-7058

Research Article

INFLUENCE OF THE COMBINATION OF PASAK BUMI ROOT AND PROPOLIS EXTRACTS ON PARASITEMIA LEVELS IN MICE INFECTED WITH PLASMODIUM BERGHEI

NUR HASANAH[1], HENDRI ASTUTY[2]*

[1]Undergraduate Program, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia. [2]Department of Parasitology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia. Email: astutiek@yahoo.com

Received: 30 September 2018, Revised: 30 September 2018, Accepted: 13 March 2019

ABSTRACT

Objective: Malaria infection remains a global concern due to increasing resistance to artemisinin-based combination therapy. This study examined the antimalarial effects of propolis extract alone and in combination with pasak bumi root extract.

Methods: In the study, 30 mice were divided into six groups including two control groups, two groups of mice treated with propolis alone at concentrations of 90 and 180 mg/kg body weight (BW), and two combination groups of mice treated with 90 or 180 mg/kg BW propolis in combination with 60 or 75 mg/kg BW pasak bumi, respectively. Plasmodium berghei 2% was injected into each mouse, and blood smears were prepared after 8 days to assess parasitemia.

Results: The results revealed no significant difference in parasitemia levels between the positive control and the two combination groups (p=0.136 and 0.289, respectively). However, superior growth inhibition (GI) results were observed in the combination groups (97.97% and 97.83%, respectively) than in the propolis monotherapy groups, whereas better outcomes were observed in the positive control group (98.63% GI) than in the propolis monotherapy groups (23.88% and 51.66%, respectively).

Conclusion: These results illustrate that combination therapy is superior to propolis monotherapy in inhibiting parasitemia.

Keywords: Pasak bumi, Propolis, Mice, Plasmodium berghei, Parasitemia, Oral.

 DOI: http://dx.doi.org/10.22159/ijap.2019.v11s1.015

INTRODUCTION

Malaria is an infectious disease caused by Plasmodium parasites and transmitted by Anopheles mosquitoes [1]. According to the World Health Organization (WHO) data, malaria was linked to 438,000 deaths globally in 2015 [2]. In Indonesia, the incidence of malaria has reached 2.9%, up from 1.9% in 2013. The incidence and prevalence of malaria are highest in Papua, East Nusa Tenggara, West Papua, Central Sulawesi, and Maluku [3].

Malaria remains prevalent due to resistance to malaria therapy [2]. In Indonesia, chloroquine is used for antimalarial therapy, but resistance to artemisinin-based combination therapy (ACT) first appeared in 2004 [4,5]. Similarly, the resistance of Plasmodium falciparum to ACT has also been detected in Cambodia, Laos, Myanmar, Vietnam, and Thailand [2]. However, the WHO continues to recommend ACT due to its efficacy [6].

At present, other alternatives for treating malaria have been investigated, such as the use of pasak bumi (Eurycoma longifolia) and propolis. Pasak bumi root has displayed antimalarial effects in both in vivo and in vitro studies [7]. In addition, the antimalarial effects of pasak bumi are stronger than those of chloroquine [8]. Research on the usefulness of propolis and its components has been widely conducted. Chalcone compounds in propolis reduce hemolysis induced by isosorbide by ≤40%, demonstrating its good anti-Plasmodium effects [9]. Recently, propolis has increasingly been studied for its antimalarial effects [10]. As antimalarial therapy, pegs can be combined with ACT and chloroquine. Pre-existing research has examined the combination of chloroquine and propolis as antimalarial therapy, but no research has assessed the combination of pasak bumi and propolis. Therefore, this study analyzed the combination of pasak bumi and propolis in the treatment of malaria [7,11].

METHODS

This in vivo study was conducted at the Try Animal Laboratory of the Ministry of Health of Indonesia, Parasitology Laboratory of FKUI, and RIK Laboratory of Depok between April 2016 and December 2016. The study subjects were healthy (active) Mus musculus (Swiss) mice (3–4 months old, 20–30 g). Mice with defects or those that died during experimentation were excluded from the analysis. The independent variables were the concentration and type of treatment given to the experimental groups. The dependent variable was the parasitemia level in mice.

In the study, mice were divided equally into six groups in cages marked with picric acid as follows:

1. Group 1: Negative control (K−) infected with 0.2 ml of Plasmodium berghei 1% without any therapy.
2. Group 2: Positive control (K+) infected with 0.2 ml of P. berghei 1% and treated with ACT 1.7 mg/kg body weight (BW) orally for the first 3 days after infection.
3. Group 3: Treatment 1 (K1) infected with 0.2 ml of P. berghei 1% and treated with oral propolis 75 mg/kg BW for 4 days.
4. Group 4: Treatment 2 (K2) infected with 0.2 ml of P. berghei 1% and treated with oral propolis 150 mg/kg BW for 4 days.
5. Group 5: Treatment 3 (K3) infected with 0.2 ml of P. berghei 1% and treated with a combination of pasak bumi 60 mg/kg BW and propolis 90 mg/kg BW daily for 4 days.
6. Group 6: Treatment 4 (K4) infected with 0.2 ml of P. berghei 1% and treated with a combination of pasak bumi 75 mg/kg BW and propolis 180 mg/kg BW daily for 4 days.

P. berghei was grown and propagated in donor mice. P. berghei obtained from the Department of Parasitology at FKUI was injected intraperitoneally into donor mice at a volume of as much as 1 ml. The

mice were then incubated until a parasitemia level of 2% was achieved as determined through blood sampling. Blood was then diluted using Roswell Park Memorial Institute at a ratio of 1:9. In total, 0.2 ml of diluted blood was injected intraperitoneally into mice. Treated mice were incubated for 24 h, after which blood was obtained daily through the tail vein without anesthesia to assess parasitemia through Giemsa staining using standard procedures [12].

Parasitemia in 1000 erythrocytes was examined using a microscope with ×1000 magnification and oil emersion. The percent parasitemia was determined using the following formula:

% Parasitemia = (Number of infected erythrocytes/Total number of erythrocytes)×100

The percent growth inhibition (GI) was calculated using Peter's 4-day suppressive test method through the following formula:

GI = ([Parasitemia K(−)−Experimental group])/(Parasitemia K[−])×100

Note: K (−) = Negative control.

The dihydroartemisinin-piperaquine (DHP) dose was determined as follows:

Human dose=Mouse dose×mouse factor/human factor.

4 mg=Mouse dose×3/37.

Mouse dose=49.3 mg/kg BW.

The DHP dose obtained using the formula was 49.3 mg/kg BW. This dose was diluted to 1 mg/ml.

One propolis tablet contains 500 mg of propolis. The crushed tablet was then diluted with distilled water to doses of 90 and 180 mg/kg BW versus the use of doses of 25, 50, and 100 mg/kg BW per day in a prior study [13].

Pasak bumi extract was generated by diluting pasak bumi powder with Aqua Dest. The available extract concentration is 2 g/50 ml water (65% active ingredient). The concentration of the extract was adjusted to the total volume administered daily using the following formula:

V1×N1=V2×N2

Formula description:
V1=Administration volume.
N1=Parasitemia concentration of donor mice.
V2=Volume of pasak bumi extract.
N2=Concentration of parasitemia desired.

RESULTS

Figure 1 presents microscopic images of erythrocytes from control and infected mice. On the 4th day, the positive control erythrocytes remain intact and healthy (Fig. 1a). Conversely, the infected parasites appeared pale (Fig. 1b).

As shown in Table 1, the parasitemia level increased each day in the negative control group. Conversely, parasitemia levels remained low in the positive control group, with the lowest level observed on day 3.

Meanwhile, parasitemia levels were lower in the propolis monotherapy groups than in the negative control group, whereas the levels were dramatically elevated versus those in the positive control group. However, a dose-dependent effect of propolis was observed.

In both combination treatment groups, parasitemia levels increased until day 1 before declining on subsequent days, with stronger antimalarial effects observed with the higher doses of pasak bumi and propolis. On day 4, the levels of parasitemia in both combination groups were similar to those of the positive control.

GI

The results for inhibition of parasitemia are summarized in Figs. 2 and 3.

The results illustrated that the greatest GI was observed in the K+ group followed by the K3 and K4 groups. Meanwhile, weaker effects were observed in the K1 and K2 groups, particularly the K1 group. Table 2 outlines the relationship between the level of parasitemia and GI from day 0 to day 4. The data illustrated that the parasitemia level was inversely proportional to GI.

Statistical analysis of parasitemia

Differences in parasitemia between days 0 and 4 were examined through a suppressive test method. These data were tested for normality using the Shapiro–Wilk test, whereas the Kruskal-Wallis test was used to identify significant differences. The results of the analysis are presented in Table 3.

Post hoc analysis of these differences was performed using the Mann–Whitney U-test as presented in Table 4.

The results illustrated significant differences between the K1 group and the K3 and K4 groups. Similarly, significant differences were noted between the K2 group and the K3 and K4 groups. However, the results

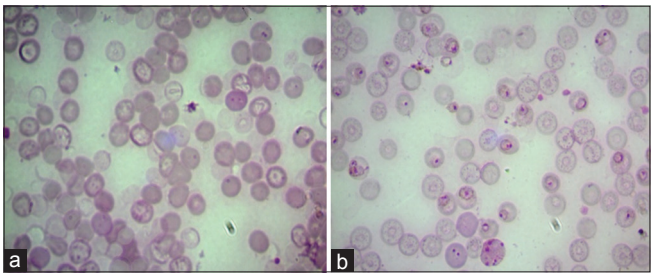

Fig. 1: Microscopic images of erythrocytes. (a) Positive control, the cells appear healthy and intact. (b) Negative control, the cells display visible signs of infection

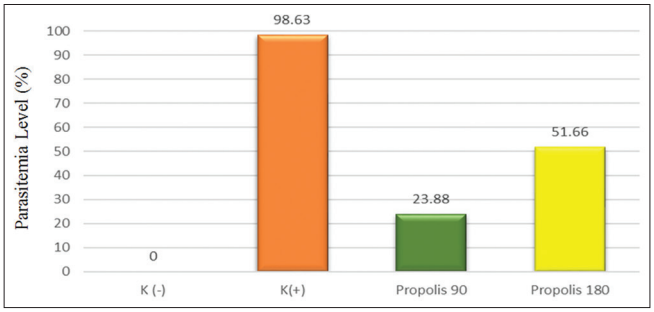

Fig. 2: Growth inhibition by propolis monotherapy

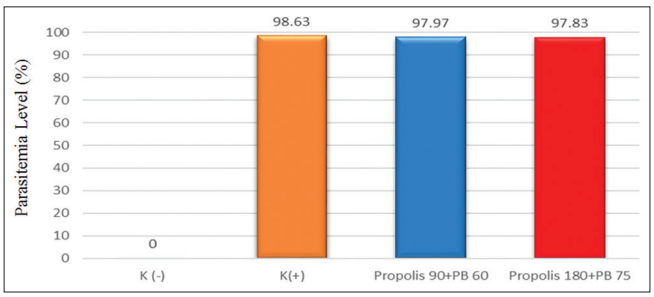

Fig. 3: Growth inhibition by the combination treatment

Table 1: Parasitemia levels

Group	n	Parasitemia level (%)					Day 4–Day 0
		Day 0	Day 1	Day 2	Day 3	Day 4	
Negative control	5	0.52	7.46	20.24	42.78	45.22	44.70
Positive control	5	0.52	1.72	0.96	0.48	0.62	0.10
Propolis 90 mg/kg BW	5	0.74	3.76	11.96	26.44	34.42	33.68
Propolis 180 mg/kg BW	5	0.64	1.34	7.6	18.04	21.86	21.22
Propolis 90 mg/kg BW+PB 60 mg/kg BW	5	0.58	5.5	4.62	1.96	0.92	0.34
Propolis 180 mg/kg BW+PB 75 mg/kg BW	5	1.08	3.46	2.54	1.52	0.98	−0.1

Parasitemia was assessed starting 24 h after Plasmodium infection, N: Number of mice per group, PB: Pasak bumi, Negative control = Mice infected without treatment, Positive control = Mice infected and treated with artemisinin combination therapy

Table 2: Relationship between parasitemia and growth inhibition

Group	n	Parasitemia levels and GI (%)									
		Day 0		Day 1		Day 2		Day 3		Day 4	
		P	GI	P	GI	P	GI	P	GI	P	GI
Negative control	5	0.52	0	7.46	0	20.24	0	42.78	0	45.22	0
Positive control	5	0.52	0.00	1.72	76.94	0.96	95.26	0.48	98.88	0.62	98.63
Propolis 90 mg/kg BW	5	0.74	−42.31	3.76	49.60	11.96	40.91	26.44	38.20	34.42	23.88
Propolis 180 mg/kg BW	5	0.64	−23.08	1.34	82.04	7.6	62.45	18.04	57.83	21.86	51.66
Propolis 90 mg/kg BW+PB 60 mg/kg BW	5	0.58	−11.54	5.5	26.27	4.62	77.17	1.96	95.42	0.92	97.97
Propolis 180 mg/kg BW+PB 75 mg/kg BW	5	1.08	−107.69	3.46	53.62	2.54	87.45	1.52	96.45	0.98	97.83

Parasitemia and growth inhibition were assessed starting 24 h after Plasmodium infection, n: Number of mice per group, P: Parasitemia level, GI: Growth inhibition, Negative control = Mice infected without treatment, Positive control = Mice infected and treated artemisinin combination therapy, PB = Pasak bumi

Table 3: Differences of parasitemia levels between days 0 and 4

Group	n	Brinkman index	p value
Positive control	5	0.00 (−0.10–0.40)	<0.05
Negative control	5	50.70 (8.30–65.40)	
Propolis 90 mg/kg BW	5	32.50 (1.30–65.00)	
Propolis 180 mg/kg BW	5	26.00 (22.70–27.60)	
Propolis 90 mg/kg BW+PB 60 mg/kg BW	5	0.30 (0.00–0.70)	
Propolis 180 mg/kg BW+PB 75 mg/kg BW	5	−0.10 (−0.70–0.80)	

Kruskal–Wallis test, PB: Pasak bumi, BW: Body weight

Table 4: Post hoc **analysis of differences in parasitemia levels between days 0 and 4**

Group	Asymp. Sig. (two-tailed)
Positive control	
Negative control	0.009
Propolis 90 mg/kg BW	0.009
Propolis 180 mg/kg BW	0.009
Propolis 90 mg/kg BW+PB 60 mg/kg BW	0.136
Propolis 180 mg/kg BW+PB 75 mg/kg BW	0.289
Negative control	
Propolis mg/kg BW	0.251
Propolis mg/kg BW	0.117
Propolis mg/kg BW+PB 60 mg/kg BW	0.009
Propolis 180 mg/kg BW+PB 75 mg/kg BW	0.009
Propolis 90 mg/kg BW	
Propolis 180 mg/kg BW	0.465
Propolis 90 mg/kg BW+PB 60 mg/kg BW	0.009
Propolis 180 mg/kg BW+PB 75 mg/kg BW	0.009
Propolis 180 mg/kg BW	
Propolis 90 mg/kg BW+PB 60 mg/kg BW	0.009
Propolis 180 mg/kg BW+PB 75 mg/kg BW	0.009
Propolis 90 mg/kg BW+PB 60 mg/kg BW	
Propolis 180 mg/kg BW+PB 75 mg/kg BW	0.141

Post hoc Mann–Whitney U-test, PB: Pasak bumi, BW: Body weight

in both combination groups were not significantly different from those of the positive control.

DISCUSSION

This study compared propolis monotherapy at two doses to combination treatment with propolis and pasak bumi. The doses of pasak bumi were based on Ridzuan's research using P. yoelli and a separate study by Adiprameswari [7,14]. The studies illustrated that pasak bumi enhanced the effects of both artemisinin and chloroquine when used in combination. Similarly, the combination of pasak bumi and propolis is also expected to provide stronger effects against parasitemia.

The effects of propolis monotherapy and combinations on parasite growth

The study results indicated that propolis alone had weaker effects on parasite growth than the positive control ACT, although the higher dose of propolis more strongly suppressed parasitemia than the lower dose. This finding is in line with research conducted by Syamsudin stating that propolis has dose-dependent effects on parasitemia. Similarly, studies conducted by Syamsudin and Olayemi also revealed that propolis monotherapy resulted in higher levels of parasitemia than treatment with chloroquine [13,15]. However, the dose of propolis should not exceed the toxic dose of 7.34 g/kg BW in mice or 50 g/ml in patients, as the therapy is ineffective above this threshold [16,17].

Propolis, when combined with pasak bumi, had longer-lasting effects against parasitemia than the positive control, leading to suppressed parasite growth on day 3. A study by Olayemi similarly identified a peak difference in activity between chloroquine and propolis, resulting in different effects [15]. The differences could also be due to the use of impure extracts. In this study, a crude propolis extract that was obtained commercially was used, and thus, the results are likely different for propolis obtained through pure extraction or diffraction [17].

The results of the study indicated that propolis and pasak bumi had synergistic effects against parasitemia. Prior research similarly demonstrated that pasak bumi was more effective when used in combination with other treatments than as monotherapy [7]. Similar outcomes have also been reported for propolis [15,17].

CONCLUSION

The combination of pasak bumi root extract and propolis had better effects against parasitemia than propolis monotherapy in mice infected with P. berghei.

CONFLICTS OF INTEREST

All authors declare that they have no conflicts of interest.

REFERENCES

1. Siciliano G, Alano P. Enlightening the malaria parasite life cycle: Bioluminescent plasmodium in fundamental and applied research. Front Microbiol 2015;6:391.
2. World Health Organization. World Malaria Report 2015. 3rd ed. Italy: World Health Organization; 2015.
3. Basic Health Research (Riskesdas). Health Profile. Jakarta: Badan Litbangkes, Ministry of Health, Republic of Indonesia; 2013.
4. Kementrian Kesehatan RI. Epidemiologi Malaria di Indonesia. Buletin Jendela Data dan Informasi Kesehatan; 2011.
5. Ministry of Health, Republic of Indonesia. Guidelines for the Treatment of Malaria in Indonesia; 2008.
6. World Health Organization. Guidelines for the Treatment of Malaria. France: World Health Organization; 2015.
7. Ridzuan MA, Sow A, Rain AN, Ilham AM, Zakiah I. *Eurycoma longifolia* extract-artemisinin combination: Parasitemia suppression of *Plasmodium yoelli* infected mice. Trop Biomed 2007;24:111-8.
8. Mohammad M, Ali MW, Ahmad A. Modelling for extraction of major phytochemical components from *Eurycoma longifolia.* J Appl Sci 2010; 10:2572-7.
9. Go ML, Liu M, Wilairat P, Rosenthal PJ, Saliba KJ, Kirk K. Antiplasmodial chalcones inhibit sorbitol-induced hemolysis of *Plasmodium falciparum* infected erythrocytes. Antimicrob Agents Chemother 2004;48:3241-5.
10. De Castro SL. Propolis biological and pharmacological activities therapeutic uses of this bee-product. ARBS Ann Rev Biomed 2001; 3:49-83.
11. Huang S, Zhang CP, Wang K, Li GQ, Hu FL. Recent advance in the chemical composition of propolis. Molecules 2014;19:19610-32.
12. Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animal. J Pharmacol Pharmacother 2010;1:87-93.
13. Anidi IU, Servinsky LE, Rentsendorj O, Stephens RS, Scott AL, Pearse DB. Cd36 and fyn kinase mediate malaria-induced lung endothelial barrier dysfunction in mice infected with *Plasmodium berghei*. PLoS One 2013;8:1-13.
14. Adiprameswari MP. Effectiveness of Oral Administration of a Combination of *Eurycoma longifolia* and Chloquine as an Antimalarial in *Plasmodium berghei* Infected Mice. [Thesis]. Faculty of Medicine, Universitas Indonesia; 2015.
15. Olayemi KI. Therapeutic potentials of Nigerian insect-propolis against the malaria parasit, *Plasmodium berghei* (*Haemosporida*: *Plasmodidae*). Am J Drug Discov Dev 2014;4:241-7.
16. Santos VR. Propolis: Alternative Medicine for the Treatment of Oral Microbial Diseases. Intech; 2012. Available from: http://www.intechopen.com/books/alternative-medicine/antifungal-activity-of-propolis-oral-clinical-studies-in-humans. [Last cited on 2016 May 28].
17. Kaewmuangmoon J, Nonthapa P, Rattanawannee A, Winayanuwattikun P, Chanchao C. Preliminary screening for various bioactivities in honey and propolis extracts from Thai bees. Eur J Med Plants 2012;2:74-92.